

Nontarget Mass Spectrometry Reveals New Perfluoroalkyl Substances in Fish from the Yangtze River and Tangxun Lake, China

Yanna Liu,[†] Manli Qian,[‡] Xinxin Ma,[§] Lingyan Zhu,[§] and Jonathan W. Martin^{*,†,||}

[†]Division of Analytical and Environmental Toxicity, Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta Canada, T6G 2G3

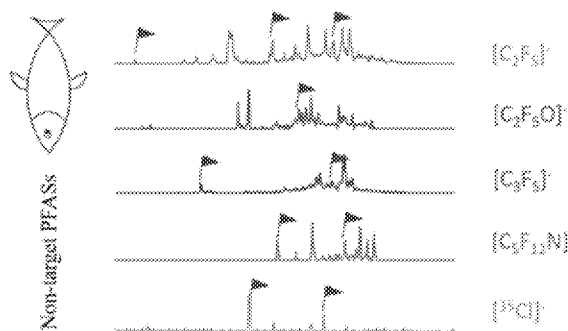
[‡]Analytical Chemistry Laboratory, Wuxi AppTec, Suzhou, China, 215104

[§]Key Laboratory of Pollution Processes and Environmental Criteria, Ministry of Education, College of Environmental Science and Engineering, Nankai University, Tianjin, China, 300071

^{||}Science for Life Laboratory, Department of Environmental Science and Analytical Chemistry, Stockholm University, Stockholm, Sweden, 10691

Supporting Information

ABSTRACT: Nontarget high-resolution mass spectrometry (Nt-HRMS) has been proven useful for the identification of unknown poly- and perfluoroalkyl substances (PFASs) in commercial products and water, but applications to biological samples are limited. China is the major PFAS-manufacturing nation; thus, here, we adapted our Nt-HRMS methods to fish collected from the Yangtze River and Tangxun Lake to discover potentially bioaccumulative PFASs in aquatic organisms destined for human consumption. In addition to traditional PFASs, over 330 other fluorinated analytes belonging to 10 classes of PFASs were detected among the pooled fish livers, including 6 sulfonate classes, 2 amine classes, 1 carboxylate class, and 1 *N*-heterocycle class. One class was detected in samples from both locations, 8 classes were detected exclusively in Tangxun Lake fish, and 1 class was detected exclusively in Yangtze River fish, 10 km downstream of a fluorochemical manufacturing site where we first reported these substances in wastewater 3 years ago. Overall, 4 of the PFAS classes (>165 analytes) are reported for the first time here. Wider monitoring and toxicological testing should be a priority for understanding the health risks posed to people and wildlife exposed to these substances.



INTRODUCTION

Many long-chain poly- and perfluoroalkyl substances (PFASs) are now globally distributed as environmental contaminants.^{1,2} Since their first discovery in the environment 18 years ago,¹ voluntary industrial phase-outs^{3–5} and domestic or international restrictions and regulations^{4–11} have been introduced to limit future emissions. However, there has been a global geographic shift of PFAS manufacturing to countries with fewer restrictions and a simultaneous shift toward alternative PFASs^{12–14} with uncertain toxicity and environmental fate. Owing to the environmental persistence of many perfluorinated compounds, there is little evidence that environmental concentrations of legacy PFASs are yet declining;¹⁵ thus, it is important to identify and prevent new alternative PFASs from spreading globally.

After the 3M Company phased out its C_8 production in North America and Europe between 2000 and 2002, developing countries (e.g., China) increased their PFAS production,¹⁶ and contamination of local environments^{17,18} and of human serum¹⁹ has followed. The link between a contaminated environment and local people is well-described

for fisheries around Tangxun Lake in China.¹⁸ Here, wastewater from an industrial region is known to have contaminated the lake water, such that it now contains very high concentrations of long chain PFASs, including perfluorooctanesulfonate (PFOS, 73–1700 ng/L) and perfluorooctanoate (PFOA, 71–1400 ng/L) as well as known alternative PFASs such as perfluorobutanesulfonate (PFBS, 2200–4500 ng/L) and perfluorobutanoate (PFBA, 1800–6300 ng/L). In the blood of fishery employees who live around this lake, and who also likely consume the fish, serum PFOS concentrations were extremely high, up to 31 000 ng/mL.²⁰ Moreover, the use of PFASs in China may have global consequences, as shown for F-53B ($ClC_6F_{12}OC_2F_4SO_3K$, CAS no. 73606-19-6), a metal plating mist suppressant chemical used only in China since the 1970s.^{21–23} F-53B is detectable in Chinese environmental^{22–24}

Received: February 9, 2018

Revised: April 5, 2018

Accepted: April 16, 2018

Published: April 16, 2018

and biological samples^{24–27} but was also recently detected in Greenland ringed seals and polar bears.²⁸

Shorter-chain PFASs and structurally modified long-chain perfluorinated acids, such as those with ether bonds or nonfluorinated carbons, are two broad categories of known alternative PFASs.¹³ Manufacturers claim that such alternatives are less bioaccumulative and “safe for their intended use”,^{29,30} but there are few publicly available toxicity studies and little environmental fate data for these. In many cases, the alternative PFASs or their predicted breakdown products do not avoid the problem of environmental persistence. For example, trifluoroacetic acid and PFBS were similarly resistant to microbial degradation as PFOS,^{31,32} and perfluoroether chains were as resistant as perfluoroalkyl chains to abiotic and biotic degradation.³³ Moreover, shorter-chain alternatives do not necessarily avoid unwanted bioaccumulative properties. Perfluorobutanesulfonamide (a PFBS precursor) was recently detected at 80 ng/g in fish in the Netherlands,³⁴ suggestive of its bioaccumulation potential.

Another concern is that unknown PFASs are now being intentionally manufactured as alternatives or unintentionally produced as byproducts and that these may already be adding to environmental contamination. At a fluorochemical manufacturing site on the Yangtze River in China, our previous work by nontarget high-resolution mass spectrometry (Nt-HRMS) revealed 36 new PFASs in wastewater,³⁵ including 3 PFAS classes that had not previously been reported. Moreover, it is generally reported that >90% of the total mass balance of organic fluorine in environmental^{36,37} and biological samples^{27,38,39} is from unknown compounds. These unknowns may be attributable to unidentified legacy PFASs or to contemporary alternative PFASs, and it is important to identify these unknowns to evaluate or mitigate risks of PFAS exposure.

Nt-HRMS techniques are valuable tools for chemical discovery, owing to their high-mass spectral resolving power, which reduce interferences, and their high mass accuracy, which enables accurate empirical formula prediction. Their successful application to PFAS discovery in water^{35,40–42} or in firefighters' serum⁴³ are already demonstrated. Here, we adapted our Nt-HRMS method for PFAS discovery in water³⁵ to the discovery of potentially bioaccumulative PFASs in fish tissue collected at two commercial fishery locations in China. Details of each chemical discovery and of related environmental and toxicological significance are discussed.

MATERIALS AND METHODS

Sample Collections. In October 2015, a commercial fisherman was recruited at each location (Figure S1) to collect fish on their regular routes. One location was on the Yangtze River, in a region approximately 10 km downstream of a wastewater treatment plant (WWTP) that services a major fluorochemical manufacturing park in Changshu, Jiangsu.³⁵ We previously identified new PFASs in influent to this WWTP, and the effluent is known to be released to the Yangtze River.⁴⁴ The other location was in Tangxun Lake in Wuhan, Hubei. This lake receives discharge from a WWTP that processes both domestic and industrial wastewater and has been identified as a contaminated water body.¹⁸

A total of three fish species were obtained at the Yangtze River site, including common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), and bighead carp (*Aristichthys nobilis*). The same three species were obtained at Tangxun Lake as well as a fourth species, white Amur bream (*Parabramis*

pekinensis). Between three and seven individual whole fish were obtained and weighed (whole-fish wet weight) for each species at each location. The whole liver was collected from each individual fish, weighed (wet liver weight), and placed into a clean polyethylene bag with changing of gloves between fish to avoid cross-contamination. Livers were shipped overnight in an ice box to Nankai University (Tianjin, China), where all liver samples and 10 g of potassium chloride granules (as quality control for possible laboratory contamination) were freeze-dried, weighed (dry liver weight; Table S1), and shipped to the University of Alberta for analysis.

Sample Extraction and Nt-HRMS Analysis. The same number of freeze-dried individual livers from the same species at each location were pooled, resulting in seven fish samples (three for the Yangtze River and four for Tangxun Lake; Table S1). All of the fish samples (one pooled sample per species at each site) with the procedural blank salts were extracted with acidified acetonitrile and purified with weak anion exchange cartridges (detailed in the Supporting Information). After successive washing with 2% formic acid, water, and methanol, the cartridges were eluted with 1% NH₄OH in methanol, and the eluents were subjected to a previously developed nontarget high-performance liquid chromatography (HPLC)-Orbitrap Nt-HRMS method for PFAS discovery.³⁵ Briefly, a combination of in-source fragmentation flagging scan, full-scan and MSⁿ experiments were used to detect and characterize unknown PFASs. Fluorinated fragment ions (e.g., [C₂F₅][–]) detected by in-source fragmentation flagging were used to flag retention times of PFAS molecular ions in full-scan mode. Exact masses (±5 ppm) of the molecular ions, isotopic patterns, and in-source fragments were used to assign empirical formulas.

For each nontarget class of PFAS homologues discovered, plausible structures were proposed based on MSⁿ analyses, and confidence levels (CL = 1–4) were assigned according to established criteria.⁴⁵ Briefly, CL = 1 represents confirmed structures by match to reference standards, CL = 2 represents probable structures by comparing to library spectra or by diagnostic evidence, CL = 3 represents tentative candidates whose possible structure can be proposed but lack sufficient information to assign an exact structure (e.g., the position of a chlorine atom on an aromatic ring), and CL = 4 is used when the unknown analyte ion can be assigned an unambiguous formula, but no structural information is available.⁴⁵

Quality Control and Quantification. Procedural blanks were injected between samples to check for background responses. Instrumental quantification limits (IQLs), method quantification limits (MQLs), absolute recoveries, absolute matrix effects and absolute total extraction efficiencies were evaluated with a mixed fish liver matrix containing equal amounts of the seven pooled fish liver samples (detailed in the Supporting Information). Legacy PFASs were quantified using authentic standards, and nontarget PFASs were semiquantified against structurally similar authentic standards as described in the Supporting Information (Table S3 in particular).

RESULTS AND DISCUSSION

Method Performance. Absolute recoveries of the solid-phase extraction method for model mass-labeled PFASs were <80% (Figure S2A) and decreased with increasing chain-length for PFCAs. The absolute total extraction efficiencies were 5–30% for all tested PFASs (Figure S2B,C). MQLs of the whole method were between 0.02–2.1 ng/g in wet liver (Table S2),

acceptable for discovering potentially bioaccumulative contaminants.

Legacy PFASs. C_4 – C_{14} PFCAs and C_4 – C_{10} PFASs were detected in all fish samples from both locations. PFOS-precursors and PFCA precursors were occasionally detected, while perfluorophosphonic acids were not detected in any sample (Table S2 and Figure S3). PFCAs were generally present at very low levels in all fish (i.e., \sum PFCAs < 46 ng/g), whereas PFASs were the major legacy PFASs detected. The Tangxun lake fish generally had higher levels of PFASs than the Yangtze River fish (i.e., \sum PFASs: 50–950 versus 7–25 ng/g, respectively), and the highest concentrations were in bream fish from Tangxun Lake. PFOS was the dominant legacy PFAS in all fish, contributing 58–72% of all legacy PFASs in the Yangtze River fish and 93–97% in Tangxun Lake fish. The exceptionally high PFOS in bream fish in Tangxun Lake (750 ng/g wet weight) is consistent with a report in which PFOS was detected at 540–1900 ng/g in crucian carp liver collected in the same lake 2 years earlier.²⁵

Nontarget PFASs. The fragmentation flagging scans of pooled fish liver extracts from Tangxun Lake and Yangtze River showed multiple F-containing fragment ion peaks (e.g., $[C_2F_5]^-$) and $[Cl]^-$ peaks (Figure 1) corresponding to

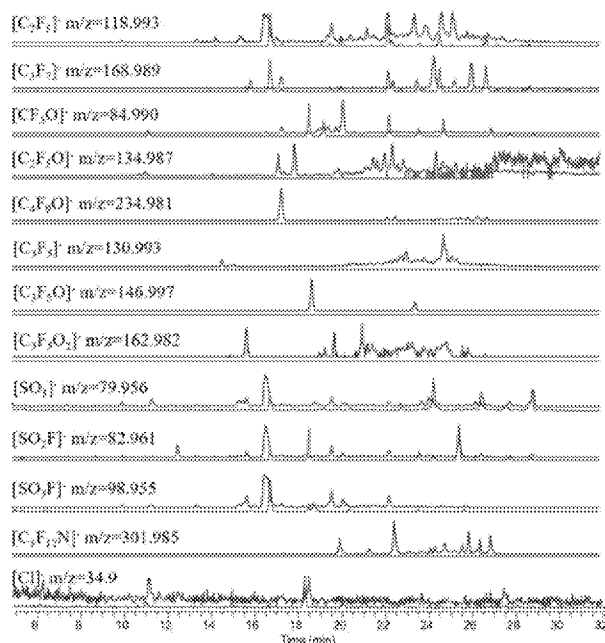


Figure 1. Extracted-ion chromatograms of fluorine-containing fragments detected by the Orbitrap mass analyzer (± 5 ppm) and of chloride fragments detected by the ion trap (± 0.5 Da). Red chromatograms are for pooled bream fish liver from Tangxun Lake, and blue chromatograms are for pooled common carp liver from the Yangtze River.

unknown PFASs. In total, the Nt-HRMS method revealed 10 homologous classes of PFASs in the 7 fish liver samples (Table 1). None of these nontarget analytes were detected in any quality control blanks, allowing us to rule out laboratory and instrumental background as their sources. Each PFAS class detected had at least six chain-length homologues, and isomers of a given homologue were commonly, but not always, observed. At each location, the homologue and isomer profiles were very similar among fish species, although minor analytes

Table 1. Nontarget PFASs Detected in Pooled Fish Liver from the Yangtze River (YR) and Tangxun Lake (TX)^a

Class No.	PFAS	Molecular Formula & Possible Structures	Chain-length range (n) [dominant n]		CL	\sum ng/g
			YR	TL		
1	terminal chlorine substituted perfluoroalkyl carboxylates	$ClC_nF_{2n-2}O_2^-$ 	8–15 [10]		3	38
2	x:2 chlorine substituted perfluoroalkyl ether sulfonates	$ClC_nF_{2n-2}SO_3^-$ 	7–14 [8]	8–10 [8]	1 & 2	88
3	cyclic or unsaturated perfluoroalkyl sulfonates	$C_nF_{2n-1}SO_3^-$ 		7–14 [12]	3	18
4	monoether-perfluoroalkyl sulfonates	$C_nF_{2n-1}SO_3^-$ 		4–12 [8]	3	120
5	diether-perfluoroalkyl sulfonates	$C_nF_{2n-1}SO_3^-$ 		6–12 [9]	3	6.9
6	enol ether/cyclic ether-carbonyl-perfluoroalkyl sulfonates	$C_nF_{2n-1}SO_4^-$ 		6–13 [11]	3	38
7	diether-unsaturated-/cyclic-ether-/carbonyl-ether-perfluoroalkyl sulfonates	$C_nF_{2n-1}SO_3^-$ 		6–13 [10]	3	10
8	perfluoroalkyl amine carboxyl esters	$C_nF_{2n-1}H_2O_4N^-$ 		13–18 [15]	4	620
9	unsaturated perfluoroalkyl amine carboxylate/carboxyl esters	$C_nF_{2n-2}O_3N^-$ 		10–15 [13]	3	74
10	perfluorinated N-heterocycles	$C_nF_{2n-3}N_2O^-$ 		9–17 [12]	3	240

^aThe range of carbon length homologues (n) detected in both locations, carbon chain length of the dominant homologue for each class [x], and the highest semi-quantified total concentration for each class in all seven fish samples (\sum ng/g in wet liver) are presented. Example structures for each class are shown (all carbons are bound to F atoms unless otherwise indicated in the structure), and associated confidence levels (CL = 1–4) are indicated.

in one fish species were sometimes absent in another due to differences in concentrations between species.

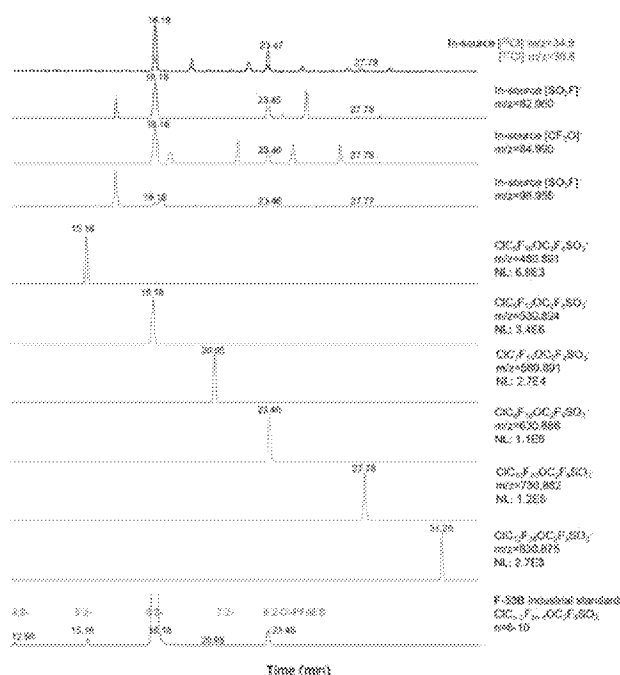
For structural elucidation of the unknown PFASs, one or two abundant homologues in each class were analyzed by MSⁿ experiments in one of the fish species that had relatively high responses, and the proposed structures are generalized for the whole class. In the following sections, each discovered PFAS class is presented with detailed discussion of the mass spectral evidence, and the novelty for each class is discussed with respect to previous literature.

Class 1: Terminal Chlorine Substituted Perfluoroalkylcarboxylates, Cl-PFCAs, $ClC_nF_{2n-2}O_2^-$, $n = 8$ –15. We previously discovered 5 classes of new PFASs in influent³⁵ to a WWTP

located ~10 km upstream of where the Yangtze River fish were collected, and a suspect screening was therefore performed in all fish samples. A single class (i.e., Cl-PFCAs) was observed in all of the Yangtze River fish (Table 1 and Figure S4.1), but none were detected in the Tangxun Lake fish. The in-source [^{35}Cl] $^-$ and [^{37}Cl] $^-$ peaks, the $[M]^-/[M+2]^- = 3:1$ isotopic pattern, and MS^2 fragmentation pattern (Figure S4.2) were consistent with the previous water study, and a terminal-chlorine-substituted PFCA structure for this class was therefore proposed (CL = 3, Table 1). Homologues with 8–15 carbons were detected, and there was only one isomer for each homologue.

This is the first ambient environmental measurement of Cl-PFCAs, and we are not aware of previous reports. Compared to our previous analysis of WWTP influent, shorter C_5 – C_7 homologues were absent in fish, while longer C_{13} – C_{15} homologues were present, likely indicating a higher bioaccumulation potential for longer-chain homologues, a well-known phenomenon among legacy PFASs.^{46–49} Moreover, the C_8 homologue that dominated the Cl-PFCA class in the wastewater³⁵ had the lowest response among all homologues in fish livers, while the C_{10} and C_{11} homologues dominated the profile in fish (Table S3 and Figure S4.1). The total estimated concentration of this class ($\sum \text{Class-1}$) was 2.7 and 5.6 times higher than the total legacy PFCA ($\sum \text{legacy-PFCAs}$) in common carp liver and silver carp, respectively (Table S3).

Class 2: x:2 Chlorine Substituted Perfluoroalkyl Ether Sulfonates, x:2 Cl-PFAESs, $\text{ClC}_n\text{F}_{2n}\text{SO}_4^-$, $n = 7–10, 12, 14$. A homologous class of nontarget analytes was detected by in-source [Cl] $^-$ that aligned with various fluorinated ion fragments: [SO_2F] $^-$, [COF_3] $^-$, and [SO_3F] $^-$ (Figure 2). These were observed in all fish samples from both locations,



after neutral loss of SO_3 . Short perfluoroalkenyl fragments (i.e., $[\text{C}_4\text{F}_7]^-$) confirmed that some of the isomers had structures with a double-bond, whereas $[\text{C}_5\text{F}_9]^-$ and $[\text{C}_6\text{F}_{11}]^-$ ions were more ambiguous, representing either longer perfluoroalkenyl moieties or five- or six-membered rings.⁵¹ Unlike all isomers in the fish, the MS^2 of authentic 4-PFECHS standard did not yield the $[\text{SO}_3]^-$ ion but exclusively the $[\text{SO}_3\text{F}]^-$ ion (Figure S6.3a); the structure for this standard is a sulfonate group attached directly to a 6-membered perfluoroalkyl ring. The absence of $[\text{SO}_3]^-$ fragment was also observed for 1-perfluoromethyl-PFOS standard (Figure S6.3b), where SO_3 is directly connected to a tertiary carbon, similar to 4-PFECHS. Thus, any perfluoroalkyl ring-containing structures in fish (e.g., possibly isomers 2 or 5) likely have a $-\text{CF}_2-$ or $-\text{C}_2\text{F}_4-$ between the sulfonate and the ring, and any branched unsaturated perfluoroalkyl sulfonate structures are likely to have the branch located at non- α -carbons (Figure S6.2). The general structure for this class was therefore assigned as cyclic or unsaturated PFSA (CL = 3; Table 1).

We also detected residual C_8 (≥ 9 isomers) and C_9 (≥ 4 isomers) cyclic or unsaturated PFSA homologues in a standard of historical commercial PFOS (denoted as "3M-PFOS" hereafter). Using retention time and MS^2 spectral matches, we confirmed that unknown isomer 2 and 9 of the C_8 homologue in fish (Figure S6.2) are the same unknown isomers in 3M-PFOS. Thus, these substances may be present in the environment as residual byproduct of historical PFOS manufacturing. In all Tangxun Lake fish samples, Σ Class 3 concentrations were estimated at $<5\%$ of Σ legacy PFSA (Figure S3 and Table S3), and C_{11} and C_{12} were the dominant homologues (Table S3).

We are unaware of any previous reports of unsaturated PFSA in any biological sample, but in total, five cyclic PFSA have been reported in waters,^{52,53} sediment,⁵¹ amphipods,⁵³ and fish.^{52,54} Among them, only 4-PFECHS^{51–55} and perfluoro-4-methylcyclohexanesulfonate⁵² were structurally confirmed. Unsaturated or cyclic PFSA isomers with 4–13 carbons were reported in a 3M PFOS standard, in aqueous film-forming foams (AFFFs),⁵⁶ and in AFFF-impacted concrete.⁵⁷

Class 4: Monoether-PFSAs, $[\text{C}_n\text{F}_{2n+1}\text{SO}_4]^-$, $n = 4–12$. Another homologous class was discovered through in-source $[\text{C}_4\text{F}_9\text{O}]^-$, which coeluted with $[\text{SO}_3]^-$, $[\text{SO}_2\text{F}]^-$, $[\text{SO}_3\text{F}]^-$, and $[\text{C}_3\text{F}_7]^-$ in all Tangxun Lake fish samples. These fragments corresponded to molecular ions with the general formula $[\text{C}_n\text{F}_{2n+1}\text{SO}_4]^-$ ($n = 4$ and $–12$; Table 1 and Figure S7.1). More than 30 partially resolved isomers were observed among all homologues in this class.

MS^2 was performed for all C_8 isomers in bighead carp sample (Figure S7.2). While $[\text{SO}_3]^-$, $[\text{SO}_3\text{F}]^-$, and perfluoroalkyl ions (e.g., $[\text{C}_3\text{F}_7]^-$) are similar to classic perfluorinated sulfonates, the high abundance of oxy-perfluoroalkyl fragments (e.g., $[\text{C}_4\text{F}_9\text{O}]^-$) suggested the presence of an ether moiety.⁵⁸ The possibility that these unknowns are perfluoroalkyl sulfates was ruled out due to absence of $[\text{C}_8\text{F}_{17}\text{O}]^-$ ^{35,59} and $[\text{SO}_4]^-$ ion.^{35,40} This class was therefore proposed as monoether-PFSAs (CL = 3; Table 1 and Figure S7.2). The $[\text{SO}_3]^-$ was absent for isomers 3 and 6, likely indicating a tertiary α -carbon (as discussed in the previous section, among all mono- CF_3 branched PFOS isomers, only α -branched PFOS produced no $[\text{SO}_3]^-$;⁶⁰ Figure S6.3).

The only previous report of monoether-PFSAs was a C_6 analyte detected in AFFF-exposed firefighter serum,⁴³ which was proposed as $\text{CF}_3\text{OC}_5\text{F}_{10}\text{SO}_3^-$ based on MS^2 spectrum.

Residual C_8 (≥ 6 isomers) and C_9 monoether-PFSAs (≥ 6 isomers) were also detected in 3M-PFOS, but none of these matched with those in fish. C_8 and C_9 homologues were the dominant homologues in all Tangxun Lake fish, and bream fish showed the highest estimated concentrations (~ 50 ng/g for both; Table S3). Compared to legacy PFSA of the same chain-length, this class was generally present at 10-fold lower concentrations (Figure S2); however, some long-chain homologues were comparable.⁶¹ Semiquantified Σ class 4 concentrations were at $<20\%$ of Σ legacy PFSA in all fish.

Class 5: Diether-PFSA, $[\text{C}_n\text{F}_{2n+1}\text{SO}_5]^-$, $n = 6–12$. Another homologous class was discovered through in-source $[\text{C}_2\text{F}_5\text{O}]^-$ in all Tangxun Lake fish. In full scans, these flags corresponded to the general formula $[\text{C}_n\text{F}_{2n+1}\text{SO}_5]^-$ ($n = 6–12$; Table 1 and Figure S8.1), similar to classic perfluoroalkyl sulfonates (e.g., PFOS) but with two additional oxygen atoms. The only possible way these oxygens can be incorporated to the structure is through ether bonds. Multiple partially resolved isomers were observed for homologues with ≥ 8 carbons, and over 25 analytes were observed for this class.

MS^2 analyses were performed for all C_8 isomers in the bream fish sample (Figure S8.2). $[\text{SO}_3]^-$, $[\text{CO}_3\text{F}]^-$, and $[\text{SO}_3\text{F}]^-$ are indicative of a sulfonate group, and the high perfluoroalkoxy $[\text{C}_n\text{F}_{2n+1}\text{O}]^-$ and dioxy $[\text{C}_n\text{F}_{2n+1}\text{O}_2]^-$ fragments provided empirical evidence to support the assignment of this class as diether-PFAs (CL = 3; Table 1).

This class has not previously been reported, but two C_7 analytes in water downstream of a fluorochemical facility were tentatively proposed as something similar: $\text{CF}_3\text{CHFOCF}_2\text{C}(\text{CF}_3)\text{OC}_2\text{F}_4\text{SO}_3^-$ and $\text{CF}_2=\text{CFOCF}_2\text{C}(\text{CF}_3)\text{OC}_2\text{F}_4\text{SO}_3^-$.⁴¹ Mono-, di-, and multiple-ether perfluoroalkyl carboxylates (e.g., $\text{C}_2\text{F}_5\text{OC}_2\text{F}_4\text{OCF}_2\text{COO}^-$) have been reported to be manufactured as alternatives to PFOA^{14,61} and were detected in water downstream of a fluorochemical facility.⁶² It is possible that the mono- (class 5) and diether-PFSAs (class 6) detected here were manufactured intentionally as PFOS alternatives. Σ Class 5 concentrations were estimated at $<2\%$ of Σ legacy PFSA in all fish (Figure S3 and Table S3).

Class 6: Enol-Ether-, Cyclic-Ether-, or Carbonyl-PFSAs, $[\text{C}_n\text{F}_{2n-1}\text{SO}_4]^-$, $n = 6–13$. Here, a homologous class was discovered by in-source $[\text{C}_3\text{F}_5]^-$ and $[\text{C}_4\text{F}_7]^-$, which coeluted with $[\text{SO}_3]^-$ and $[\text{SO}_3\text{F}]^-$ in all Tangxun Lake fish. In full scans, these fragments corresponded to the general formula $[\text{C}_n\text{F}_{2n-1}\text{SO}_4]^-$ ($n = 6–13$; Table 1 and Figure S9.1). More than 40 partially resolved isomers were observed among all homologues.

MS^2 was performed for all C_8 isomers in the bream fish (Figure S9.2). $[\text{SO}_3]^-$, $[\text{SO}_2\text{F}]^-$, and $[\text{SO}_3\text{F}]^-$ indicated an underlying PFSA structure. Neutral $\text{C}_n\text{F}_{2n}\text{O}$ loss (e.g., the production of $\text{C}_7\text{F}_{13}\text{SO}_3^-$ and $\text{C}_6\text{F}_{11}\text{SO}_3^-$) and perfluoroalkenyl fragments were commonly observed. This, along with DBE = 1, allows three feasible structures with CL = 3 (Table 1). The first are cyclic ether-PFSAs. Neutral CH_2O loss is characteristic of cyclic ethers in electron ionization⁶³ and collision-activated dissociation⁶⁴ in gas chromatography–mass spectrometry. Second are unsaturated ether-PFSAs. Perfluoroalkoxy fragments are usually expected for perfluoroalkyl ethers.^{41,58} However, no such fragments were observed for any C_8 - isomer, possibly indicating an enol ether structure in which the existence of a nearby double bond may have reduced production of the perfluoroalkoxy fragment. The third are carbonyl-PFSAs. Fragments $\text{C}_6\text{F}_{11}\text{SO}_3^-$ for isomer 7 and 9 can be formed via cleavage of a $\text{CF}_3\text{C}(\text{O})-$ moiety, while $\text{C}_7\text{F}_{13}\text{SO}_3^-$

for isomer 2–6 and 8 may be due to a terminal carbonyl fluoride (i.e., an acid fluoride). However, we believe it unlikely that such a compound would be hydrolytically stable under our alkali mobile-phase condition, not to mention persistent enough to be present in fish livers. A carbonyl fluoride compound may also not have adsorbed to the WAX cartridge used for sample purification.

Residual $C_8F_{15}SO_4^-$ (6 isomers) and $C_9F_{17}SO_4^-$ (3 isomers) were also detected in 3M-PFOS. By retention and MS^2 matches, isomers 2, 5, and 8 of the C_8 homologue in fish samples are likely of the same chemical identity as three C_8 isomers in 3M-PFOS. This class could therefore be byproducts in historical PFSA production or perhaps are manufactured intentionally as alternatives to PFOS. Semi-quantified Σ class 7 concentrations were at 1–6% of Σ legacy PFASs in all fish (Figure S3 and Table S3).

Cyclic ether-PFASs have not previously been reported. A C_7 analyte in water downstream of a fluorochemical facility was proposed as an enol ether-perfluoroalkyl sulfonate ($CF_2=CFOCF_2C(CF_3)OC_2F_4SO_3^-$, as discussed in the section on class 5) but only based on its exact mass.⁴¹ Based on MS^2 spectra, a homologous class of compounds (C_4 – C_{13}) detected in AFFF-impacted concrete⁵⁷ and a C_8 analyte detected in AFFF-exposed firefighter serum were proposed as ketone-PFASs.

Class 7: Diether-Unsaturated-, Cyclic-Ether-, or Carbonyl-Ether-PFASs, $[C_nF_{2n-1}SO_3]^-$, $n = 6$ –13. A homologous class of analytes was flagged by in-source $[C_3F_5O]^-$ and $[C_3F_5O_2]^-$, which coeluted with $[SO_3]^-$, $[SO_3F]^-$, $[C_2F_5]^-$, and $[C_3F_7]^-$, in all Tangxun Lake fish. These flags corresponded to molecular ions with the general formula $[C_nF_{2n-1}SO_3]^-$ ($n = 6$ –13; Table 1 and Figure S10.1). This formula is similar to that of class 6 analytes but with one additional oxygen atom. More than 30 partially resolved isomers were observed among all homologues.

MS^2 analysis was performed for all C_{10} isomers in silver carp (Figure S10.2). The $[SO_3]^-$ and $[SO_3F]^-$ detected for all isomers are characteristic of PFASs. Similar to class 6, the neutral loss of $[C_nF_{2n}O]$ and perfluoroalkenyl ions were observed for all isomers, indicating the same three feasible functionalities as above. The high $[C_2F_5O]^-$ fragment for isomer 1–3, as observed for classes 4 and 5, indicates an additional terminal ethyl ether (for example, structures for isomer 2 in Figure S10.2), while the absence of an obvious perfluoroalkoxy fragment for isomers 4 and 5 may indicate an internal ether structure (for example, the structures for isomer 4 in Figure S10.2). Thus, diether-unsaturated-, cyclic-ether-, or carbonyl-ether-PFASs are all possible for this class (CL = 3; Table 1).

This class was not detected in the historical 3M-PFOS standard. In fish, most homologues were estimated at <1 ng/g, except in bream fish, in which the dominant C_{10} homologue was present at ~3.0 ng/g, and semiquantified Σ class 7 concentrations were at <2% of Σ legacy PFASs (Table S3 and Figure S3). The only previous report of a chemical belonging to this class was by Strynar et al.,⁴¹ who proposed a C_7 analyte detected in water as diether-unsaturated PFSA based only on its exact mass (as discussed in classes 5 and 6).

Class 8: Perfluoroalkyl Amine Carboxyl Esters, $[C_nF_{2n-6}H_6O_6N]^-$, $n = 13$ –16. For this class of analytes, discovery was initially for a series of small N-containing ions that we later realized were fragments of larger precursor molecules, which dissociated even under the soft ionization

conditions of our full scan mode. Here, the discovery process is described sequentially, from smallest to largest ions, as it is instructive for characterization of the analyte structures.

A homologous series of ions, with the general formula of $[C_nF_{2n+2}N]^-$ ($n = 5$ –12), was initially discovered by in-source $[C_3F_7]^-$ in all Tangxun Lake fish with many isomers observed for each homologue (Table 1 and Figure S11.1). Structural characterization of the C_5 and C_8 homologues suggested a perfluoroalkyl amine structure for these ions (Figure S11.2). Although the branching of the perfluoroalkyl chain could possibly have given rise to the high number of isomers, the identical MS^2 profiles, especially for those of low-carbon-chain homologues (e.g., $C_5F_{12}N^-$), raised the possibility that these analytes may actually be identical or very similar fragments coming from larger precursor molecules.

Manual searching for higher molecular weight precursors was performed at the same retention times, and the first class of plausible precursor ions had the formula $[C_nF_{2n}O_2N]^-$ ($n = 9$ –16; Figure S11.3 and Table 1). Each $[C_nF_{2n}O_2N]^-$ homologue was composed of multiple isomeric peaks, and each peak had at least one corresponding $[C_nF_{2n+2}N]^-$ peak at the same retention time (shaded peaks in Figure S11.3), consistent with these being precursor ions. This was confirmed by MS^2 fragmentation of all $C_{12}F_{24}O_2N^-$ isomers (Figure S11.4a) as well as the major isomer of the C_{10} – C_{13} homologues (Figure S11.4b). The neutral loss of $C_nF_{2n-2}O_2$ ($n = 4$ –6) was commonly observed for the C_{10} – C_{13} homologues, corresponding to either a perfluorocarboxylate or carboxyl ester moiety (Figure S11.4).

Continued manual search for even higher molecular weight precursors revealed two more homologous classes with the formulas $[C_nF_{2n-2}H_2O_5N]^-$ ($n = 10$ –17; Figure S11.5) and $[C_nF_{2n-6}H_6O_6N]^-$ ($n = 13$ –16, Figure S11.6). All $[C_nF_{2n-2}H_2O_5N]^-$ ions had at least a corresponding $[C_nF_{2n}O_2N]^-$ peak at the same retention time (shaded peaks in Figure S11.3), and all $[C_nF_{2n-6}H_6O_6N]^-$ ions had at least a corresponding $[C_nF_{2n-2}H_2O_5N]^-$ peak at the same retention time (shaded peaks in Figure S11.5), indicating these as precursors.

Collectively, these data suggest that the analytes actually present in fish livers are isomers and homologues of $[C_nF_{2n-6}H_6O_6N]^-$, while $[C_nF_{2n-2}H_2O_5N]^-$, $[C_nF_{2n}O_2N]^-$, and $[C_nF_{2n+2}N]^-$ are fragments formed spontaneously in the source of the instrument. Neither $[C_nF_{2n-2}H_2O_5N]^-$ nor $[C_nF_{2n-6}H_6O_6N]^-$ homologues could be successfully selected in MS^2 analysis, making the structures uncertain (CL = 4; Table 1). Nevertheless, based on characterization of the $[C_nF_{2n}O_2N]^-$ fragments, a general core structure of perfluoroalkyl amine carboxyl esters is likely for the $[C_nF_{2n-6}H_6O_6N]^-$ homologues. The C_{15} homologue eluting at 24.37 min was taken as an example, and its sequential fragmentation pathway to $[C_{13}F_{24}H_2O_5N]^-$, to $[C_{12}F_{24}O_2N]^-$, and eventually to $[C_8F_{18}N]^-$ was proposed (Figure S11.7).

The number of analytes detected for this $[C_nF_{2n-6}H_6O_6N]^-$ class was estimated by counting the total number of different retention times for the $[C_nF_{2n+2}N]^-$ fragments, and at least 50 $[C_nF_{2n-6}H_6O_6N]^-$ analytes could be confirmed in fish livers. Semi-quantification for this class was performed using a PFOS calibration curve, and the concentration was calculated by adding up $[C_nF_{2n-6}H_6O_6N]^-$ and all 3 classes of fragments. Thus, the class 8 analytes were present at >10 ng/g in all 4 fish species (18–65% of Σ legacy PFSA concentrations) and reached ~630 ng/g in bream fish, comparable to the PFOS concentration (Table S3 and Figure S3).

Simple perfluoroalkyl amines are marketed for use in electronics testing and heat transfer^{65,66} and as artificial oxygen carriers for medical purposes.^{67–69} Perfluoroalkyl amines are also among the starting materials for manufacturing fluoropolymers (e.g., polyurethanes and polyamides⁶⁶). The only previous report of a perfluoroamine was perfluorotributylamine, which is among the 3M Fluorinert products^{65,70,71} and was detected in urban air in Toronto.⁷² It is notable that perfluorotributylamine has an atmospheric lifetime of 500 years and has “the highest radiative efficiency of any compound detected”.⁷²

Class 9: Unsaturated Perfluoroalkyl Amine Carboxylates or Carboxyl Esters, $[C_nF_{2n-2}NO_2]^-$, $n = 10–15$. Another homologous class with the general formula $[C_nF_{2n}N]^-$ ($n = 6–15$) was discovered through in-source $[C_3F_5]^-$ in all Tangxun Lake fish (Figure S12.1). This formula is similar to class 8 $[C_nF_{2n+2}N]^-$ ions but with two fewer F atoms in the perfluoroalkylamine structure, owing to a double bond or ring. The unsaturated perfluoroalkyl amine structure was confirmed by MS² experiments with all C₈ isomers (CL = 3; Figure S12.2). Manual searching for possible precursors revealed one homologous class of ions having the general formula $[C_nF_{2n-2}NO_2]^-$ ($n = 10–15$, class 9; Figure S12.3), similar to the class 8 $[C_nF_{2n}NO_2]^-$ fragments. MS² analyses confirmed the general structure for this class as unsaturated perfluoroalkyl amine carboxylates or carboxyl esters (CL = 3; Figure S12.4).

The total number of analytes detected for class 9 $[C_nF_{2n-2}NO_2]^-$ was estimated by first counting the total number of different retention times of the $[C_nF_{2n}N]^-$ fragments (Figure S12.1) and then subtracting those in which class 8 $[C_nF_{2n+2}N]^-$ fragments were also coeluting (Figure S11.1) due to the possibility that an unsaturated $[C_nF_{2n-2}NO_2]^-$ can fragment to both saturated $[C_nF_{2n+2}N]^-$ and unsaturated $[C_nF_{2n}N]^-$. For example, $[C_{12}F_{22}NO_2]^-$ eluting at 22.80 min produced $[C_5F_{12}N]^-$ and $[C_7F_{14}N]^-$ in MS² (Figure S12.4), and $[C_5F_{12}N]^-$ and a $[C_7F_{14}N]^-$ were also detected in full scans at the same retention time (Figures S11.1 and S12.1). The semiquantified concentration for class 9 analyte was $\sim 10\times$ lower than for class 8 (Table S3 and Figure S3).

Class 10: Perfluorinated N-Heterocycles, $[C_nF_{2n-3}N_2O]^-$, $n = 9–18$. Finally, a homologous series of analytes was discovered by in-source $[C_3F_5O]^-$ and $[C_3F_7]^-$ in all Tangxun Lake fish, and these flags corresponded to compounds with the general formula $[C_nF_{2n-3}N_2O]^-$ ($n = 9–17$; Table 1 and Figure S13.1). Multiple isomers were observed for most homologues, and more than 40 analytes were detected in this class.

Structural elucidation was performed by MS², MS³, and MS⁴ fragmentation of the C₉ homologue (Figure S13.2a). Unlike any of other classes characterized above, here, radical loss and formation was commonly observed, indicating an aromatic moiety.^{73–76} The DBE = 3 indicates a 5-member heteroaromatic moiety: furan, pyrrole, pyrazole, imidazole, oxazole, and isoxazole. The loss of $\bullet OCF_3$ confirmed that oxygen was not in the ring, therefore excluding furans, oxazoles, and isoxazoles. Azo-compounds ($-N=N-$) lose N₂ in negative mode,⁷⁷ and the absence of such fragmentation narrowed down the possible core structures to either pyrroles or imidazoles.

The loss of $\bullet C_4F_9$ (i.e., $m/z = 436.98 \rightarrow 217.99$) and of C_4F_{10} ($m/z = 436.98 \rightarrow 198.99$) suggested a C₄F₉ substituent, based on the analogy that $\bullet CH_3$ and CH₄ losses are observed for isopropyl-substituted phenyls.⁷⁶ Losses of $\bullet CF_3$ ($m/z = 436.98 \rightarrow 367.97$ and $217.99 \rightarrow 149.00$), of $\bullet OCF_3$ ($m/z =$

$217.99 \rightarrow 133.00$) and of $(\bullet CF_3 + CO)$ ($m/z = 217.99 \rightarrow 121.00$) indicate a CF₃O substituent based on analogy that CH₃O substituted aromatic compounds lose $\bullet CH_3$, $\bullet OCH_3$,^{77–79} and $(\bullet CH_3 + CO)$.^{76,77,79,80} Given these two likely substituents, the most likely core structure is a substituted perfluorinated imidazole-(CF₃O)(C₄F₉)(CF₃), which is supported by MS/MS experiments (Figure S13.2). Given uncertainties and the number of isomers, the structure for this class of substances is generally proposed as perfluorinated N-heterocycles (CL = 3; Table 1).

It is noticeable that isomers for each homologue eluted as two clusters, with one cluster eluting much later (Figure S13.1). To examine the structural differences between the two clusters, MS²–MS⁵ were performed for all C₁₂ isomers, and similar fragmentation patterns were observed (Figure S13.3). The very different retention times for the two clusters may be due to different core structures (e.g., pyrrole versus imidazole) or that at least one cluster was in-source fragments from larger precursors. However, no precursor ions were discovered.

We are not aware of any previous reports of perfluorinated pyrroles/imidazoles. Fluoropyrrole derivatives have been used in pharmaceutical applications^{81–83} and as insecticides (e.g., chlorofenapyr⁸⁴), and fluorinated imidazoles are useful reagents in organic chemistry,⁸⁵ but all of these examples are not highly fluorinated. Perfluoroalkyl imidazoles are potent angiotensin II antagonists,⁸⁶ but no such commercialized drug is known. The estimated concentrations of each homologue was usually < 1 ng/g in most fish (Table S3). However, the dominant C₁₂ homologue was estimated at 3.8–210 ng/g, 8–28% of PFOS concentrations in the same sample.

SIGNIFICANCE

The in-source fragmentation flagging Nt-HRMS method,³⁵ first developed for water analysis, was shown here to be a powerful tool for unknown PFAS discovery in a complex biological matrix. The method has the potential for discovering any class of compound that fragments to produce characteristic ions in the source. Here, in addition to perfluoroalkyl (e.g., $[C_2F_5]^-$) and perfluoroalkene fragment ions (e.g., $[C_3F_5]^-$), perfluoroether (e.g., $[C_2F_5O]^-$), perfluoroamine (e.g., $[C_3F_7N]^-$), chloride (e.g., $[Cl]^-$), and S-containing fragment ions (e.g., $[SO_3F]^-$) were used to flag unknown PFASs. As shown for most discovered PFAS classes, the method benefits tremendously from liquid chromatography to separate any isomers prior to structural elucidation. In this way, a much-greater number of compounds were discovered relative to what would have been observed by direct infusion. Moreover, structural elucidation would have led to inaccuracies without chromatographic separation.

In total, over 330 nontarget PFAS analytes were detected in pooled fish liver. Except for 6:2 Cl-PFAS²⁵ (class 2) and 4-PFECHS^{52,54} (class 3), all nontarget analytes are reported in fish for the first time. Considering all previous PFAS studies in human, environmental or industrial, and commercial standards, more than 165 analytes belonging to 4 PFAS classes (classes 5, 8, 9, and 10) are reported for the first time. For the 6 PFAS classes that were previously reported in other samples, new homologues and isomers were detected here in fish, including C₁₃–C₁₅ class 1 (3 analytes); C₇, C₉, and C₁₄ class 2 (3 analytes); C₁₄ class 3 (> 2 analytes); C₄ and C₅ and C₁₀–C₁₂ class 4 (> 15 analytes); C₁₀–C₁₃ class 6 (~ 30 analytes); and C₆ and C₈–C₁₃ class 7 (~ 30 analytes).

The chain lengths of PFASs detected ranged from C₄ to C₁₈. Among the 73 total homologues, 61 homologues had at least 8 carbons, showing the dominance of long-chain PFASs in these fish. This is not likely biased by the analytical method because the extraction showed higher absolute recoveries for mass-labeled shorter-chain legacy PFCAs and PFSA than for longer-chain homologues (Figure S2), and the HPLC method was capable of retaining PFASs as short as C₄. The small number of shorter-chain PFAS detected is most likely attributable to their lower bioaccumulation potentials or lower emission in these two regions.

The semi-quantified total concentration for each PFAS class was calculated to aid future research prioritization (Figure S3). In the Yangtze River fish, classes 1 and 2 were present at similar levels in each species. In fish from Tangxun Lake, classes 4, 8, and 10 were the three dominant PFAS classes. Among the four fish species in Tangxun Lake, bream fish showed much higher concentrations for all detected PFASs than those in other species. This may be attributable to different feeding habits because bream are omnivorous whereas carp are filter-feeders.

Compared with legacy PFASs, the newly identified PFASs have monochlorine substitution, double bonds or rings, ether bonds, and carbonyl or amine functional groups. Only ketone (i.e., carbonyl) and ether-PFASs are known to have been produced intentionally as alternative PFASs.¹⁴ However, ether-PFASs (and maybe carbonyl-PFASs) were also detected as byproducts in historical 3M-PFOS. It is therefore difficult to know if any of the nontarget PFASs detected here are from historical or contemporary production. Particularly for Tangxun Lake, it would seem prudent to examine current wastewater releases for evidence of the many PFASs identified here. This may help to minimize future exposure around this contaminated water body.

Between the two sites examined, there was a clear difference in the types of PFASs detected. With the exception of class 2, detected in both locations, 8 classes were exclusively discovered at Tangxun Lake, and 1 class was only detected in the Yangtze River (Figure S3). This most likely indicates that the sources of contamination were from unique point sources (e.g., industrial wastewater), but it should also be kept in mind that we are comparing fish from a lake to fish from a river, in which residence times, dilution, and primary productivity are different. Future monitoring of water from more regions of China are warranted to understand the true spatial distribution and sources of all chemicals identified here.

The discovery of over 330 nontarget PFASs and predominantly long-chain PFASs in fish demonstrates bioavailability and is suggestive of a measurable bioaccumulation potential for these compounds or their precursors, whose environmental fate has otherwise not been studied. Given that all samples were from fish destined for fish markets and human consumption, the current findings in fish livers raise immediate questions about exposure and health risks to exposed people. Fish muscle was not analyzed in the current study, and the extent of human exposure related to it remains unknown.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b00779.

Additional details on chemicals and reagents, sample preparation, instrumental analysis, and data presentation.

Tables showing sample-weight data and PFAS concentration. Figures showing fish-collection sites, extraction recoveries, PFAS concentrations, class 1–10 PFAS chromatograms, and MSⁿ spectra. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (46)-072-146-2773; e-mail: jon.martin@aces.su.se.

ORCID

Yanna Liu: 0000-0001-7893-9876

Lingyan Zhu: 0000-0001-9318-7940

Jonathan W. Martin: 0000-0001-6265-4294

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The research was supported by the Natural Sciences and Engineering Research Council (NSERC Discovery, JW Martin, grant no. RGPIN-2016-06487). Alberta Health are acknowledged for supporting daily activities in the Division of Analytical and Environmental Toxicology. L.Z. acknowledges the Natural Science Foundation of China (grant no. 21737003). Y.L. acknowledges the China Scholarship Council and the Killam Trust for supporting her scholarship. Dr. Amila De Silva (Environment and Climate Change Canada) is thanked for providing the 4-PFECHS standard, and Dr. Lisa D'Agostino (Stockholm University) is thanked for commenting on an early draft.

■ REFERENCES

- (1) Giesy, J. P.; Kannan, K. Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **2001**, *35* (7), 1339–42.
- (2) Martin, J. W.; Asher, B. J.; Beesoon, S.; Benskin, J. P.; Ross, M. S. PFOS or PreFOS? Are perfluorooctane sulfonate precursors (PreFOS) important determinants of human and environmental perfluorooctane sulfonate (PFOS) exposure? *J. Environ. Monit.* **2010**, *12* (11), 1979–2004.
- (3) USEPA. EPA and 3M Announce Phase-out of PFOS. <https://yosemite.epa.gov/opa/admpress.nsf/0/33aa946e6cb11f35852568e1005246b4> (accessed May 13, 2016).
- (4) USEPA. 2010/2015 PFOA Stewardship Program. <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program> (accessed Sept 10, 2015).
- (5) ECCC. 5-year Environmental Performance Agreement. <http://ec.gc.ca/epe-epa/default.asp?lang=En&n=AE06B51E-1> (accessed Sept 23, 2016).
- (6) EU. Amending for the 30th Time Council Directive 76/769/EEC on the Approximation of the Laws, Regulations and Administrative Provisions of the Member States Relating to Restrictions on the Marketing and Use of Certain Dangerous Substances and Preparations: Perfluorooctane Sulfonates; European Parliament and the Council of the European Union (EU): Brussels, Belgium, 2006; pp 372–34.
- (7) ECHA. Pentadecafluorooctanoic Acid (PFOA) as A Substances of Very High Concern Because of Its CMR and PBT Problems; European Chemicals Agency (ECHA): Helsinki, Finland, 2013.
- (8) NEPA. Forbidding PFOA in Norwegian Consumer Products. <http://www.miljodirektoratet.no/no/Nyheter/Nyheter/Old-klif/2013/juni-2013/Forbyr-PFOA-i-norske-forbrukerprodukter/> (accessed July 25, 2016).
- (9) ECCC. Regulations Amending the Prohibition of Certain Toxic Substances Regulations. <http://www.ec.gc.ca/lcpe-cepa/eng/regulations/detailReg.cfm?intReg=226> (accessed July 21, 2017).
- (10) USEPA. Significant New Use Rules: Long-Chain Perfluoroalkyl Carboxylate and Perfluoroalkyl Sulfonate Chemical Substances.

<https://www.regulations.gov/document?D=EPA-HQ-OPPT-2013-0225-0001> (accessed May 25, 2017).

(11) ECHA. Candidate List of Substances of Very High Concern for Authorisation. <https://echa.europa.eu/candidate-list-table> (accessed June 2, 2017).

(12) Holt, R. Alternatives to Long-chains. FluoroCouncil: The Global Industry Council for FluoroTechnology. <https://www.oecd.org/env/ehs/risk-management/47651662.pdf> (accessed March 20, 2017).

(13) OECD. *Synthesis Paper on Per- and Polyfluorinated Chemicals (PFCs)*; Organisation for Economic Cooperation and Development (OECD): Paris, France, 2013.

(14) Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFASs) and their potential precursors. *Environ. Int.* **2013**, *60*, 242–8.

(15) Land, M.; de Wit, C. A.; Bignert, A.; Cousins, I. T.; Herzke, D.; Johansson, J. H.; Martin, J. W. What is the effect of phasing out long-chain per- and polyfluoroalkyl substances on the concentrations of perfluoroalkyl acids and their precursors in the environment? A systematic review. *Environmental Evidence* **2018**, *7* (1), 4.

(16) Lim, T. C.; Wang, B.; Huang, J.; Deng, S.; Yu, G. Emission inventory for PFOS in China: review of past methodologies and suggestions. *Sci. World J.* **2011**, *11*, 1963–80.

(17) Chen, C.; Lu, Y.; Zhang, X.; Geng, J.; Wang, T.; Shi, Y.; Hu, W.; Li, J. A review of spatial and temporal assessment of PFOS and PFOA contamination in China. *Chem. Ecol.* **2009**, *25* (3), 163–177.

(18) Zhou, Z.; Liang, Y.; Shi, Y.; Xu, L.; Cai, Y. Occurrence and transport of perfluoroalkyl acids (PFAAs), including short-chain PFAAs in Tangxun Lake, China. *Environ. Sci. Technol.* **2013**, *47* (16), 9249–57.

(19) Calafat, A. M.; Wong, L. Y.; Kuklenyik, Z.; Reidy, J. A.; Needham, L. L. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. *Environ. Health Perspect.* **2007**, *115* (11), 1596–602.

(20) Zhou, Z.; Shi, Y.; Vestergren, R.; Wang, T.; Liang, Y.; Cai, Y. Highly elevated serum concentrations of perfluoroalkyl substances in fishery employees from Tangxun lake, China. *Environ. Sci. Technol.* **2014**, *48* (7), 3864–74.

(21) Shanghai Guangming Electroplating Plant; Shanghai Institute of Organic Chemistry at Chinese Academy of Sciences Jiangsu Taizhou Electrochemical Plant. Preparation of F-53 and its application in chrome mist suppression. Shanghai Institute of Organic Chemistry at Chinese Academy of Sciences (SIOCCAS). *Mater. Prot.* **1976**, *3* (3), 27.

(22) Ruan, T.; Lin, Y.; Wang, T.; Liu, R.; Jiang, G. Identification of novel polyfluorinated ether sulfonates as PFOS alternatives in municipal sewage sludge in China. *Environ. Sci. Technol.* **2015**, *49* (11), 6519–27.

(23) Wang, S.; Huang, J.; Yang, Y.; Hui, Y.; Ge, Y.; Larssen, T.; Yu, G.; Deng, S.; Wang, B.; Harman, C. First report of a Chinese PFOS alternative overlooked for 30 years: its toxicity, persistence, and presence in the environment. *Environ. Sci. Technol.* **2013**, *47* (18), 10163–70.

(24) Liu, W.; Qin, H.; Li, J.; Zhang, Q.; Zhang, H.; Wang, Z.; He, X. Atmospheric chlorinated polyfluorinated ether sulfonate and ionic perfluoroalkyl acids in 2006 to 2014 in Dalian, China. *Environ. Toxicol. Chem.* **2017**, *36* (10), 2581–86.

(25) Shi, Y.; Vestergren, R.; Zhou, Z.; Song, X.; Xu, L.; Liang, Y.; Cai, Y. Tissue Distribution and Whole Body Burden of the Chlorinated Polyfluoroalkyl Ether Sulfonic Acid F-53B in Crucian Carp (*Carassius carassius*): Evidence for a Highly Bioaccumulative Contaminant of Emerging Concern. *Environ. Sci. Technol.* **2015**, *49* (24), 14156–65.

(26) Pan, Y.; Zhu, Y.; Zheng, T.; Cui, Q.; Buka, S. L.; Zhang, B.; Guo, Y.; Xia, W.; Yeung, L. W.; Li, Y.; Zhou, A.; Qiu, L.; Liu, H.; Jiang, M.; Wu, C.; Xu, S.; Dai, J. Novel chlorinated polyfluorinated ether sulfonates and legacy per-/polyfluoroalkyl substances: placental

transfer and relationship with serum albumin and glomerular filtration rate. *Environ. Sci. Technol.* **2017**, *51* (1), 634–644.

(27) Shi, Y.; Vestergren, R.; Xu, L.; Zhou, Z.; Li, C.; Liang, Y.; Cai, Y. Human exposure and elimination kinetics of chlorinated polyfluoroalkyl ether sulfonic acids (Cl-PFESAs). *Environ. Sci. Technol.* **2016**, *50* (5), 2396–404.

(28) Gebbink, W. A.; Bossi, R.; Riget, F. F.; Rosing-Asvid, A.; Sonne, C.; Dietz, R. Observation of emerging per- and polyfluoroalkyl substances (PFASs) in Greenland marine mammals. *Chemosphere* **2016**, *144*, 2384–91.

(29) Bowman, J. S. Fluorotechnology is critical to modern life: the FluoroCouncil counterpoint to the Madrid Statement. *Environ. Health Perspect.* **2015**, *123* (5), A112–3.

(30) Bowman, J. S. Response to "Comment on 'Fluorotechnology Is Critical to Modern Life: The FluoroCouncil Counterpoint to the Madrid Statement'". *Environ. Health Perspect.* **2015**, *123* (7), A170–1.

(31) Sibley, P. K.; Ellis, D. A.; Fineberg, N. A.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G.; Hanson, M. L.; Shahid, T. The fate and persistence of trifluoroacetic and chloroacetic acids in pond waters. *Chemosphere* **2001**, *42* (3), 309.

(32) Ochoa-Herrera, V.; Field, J. A.; Luna-Velasco, A.; Sierra-Alvarez, R. Microbial toxicity and biodegradability of perfluorooctane sulfonate (PFOS) and shorter chain perfluoroalkyl and polyfluoroalkyl substances (PFASs). *Environ. Sci. Process. Impacts* **2016**, *18* (9), 1236–1246.

(33) Wang, Z.; Cousins, I. T.; Scheringer, M.; Hungerbühler, K. Hazard assessment of fluorinated alternatives to long-chain perfluoroalkyl acids (PFAAs) and their precursors: status quo, ongoing challenges and possible solutions. *Environ. Int.* **2015**, *75*, 172–9.

(34) Chu, S.; Letcher, R. J.; McGoldrick, D. J.; Backus, S. M. A new fluorinated surfactant contaminant in biota: perfluorobutane sulfonamide in several fish species. *Environ. Sci. Technol.* **2016**, *50* (2), 669–75.

(35) Liu, Y.; Pereira, A. D. S.; Martin, J. W. Discovery of C5-C17 poly- and perfluoroalkyl substances in water by in-line SPE-HPLC-Orbitrap with in-source fragmentation flagging. *Anal. Chem.* **2015**, *87* (8), 4260–8.

(36) Tan, B.; Wang, T.; Wang, P.; Luo, W.; Lu, Y.; Romesh, K. Y.; Giesy, J. P. Perfluoroalkyl substances in soils around the Nepali Koshi River: levels, distribution, and mass balance. *Environ. Sci. Pollut. Res.* **2014**, *21* (15), 9201–11.

(37) Wang, P.; Wang, T.; Giesy, J. P.; Lu, Y. Perfluorinated compounds in soils from Liaodong Bay with concentrated fluorine industry parks in China. *Chemosphere* **2013**, *91* (6), 751–7.

(38) Loi, E. I. H.; Yeung, L. W. Y.; Taniyasu, S.; Lam, P. K. S.; Kannan, K.; Yamashita, N. Trophic magnification of poly- and perfluorinated compounds in a subtropical food web. *Environ. Sci. Technol.* **2011**, *45* (13), 5506–13.

(39) Taniyasu, S.; Senthikumar, K.; Yamazaki, E.; Yeung, L. W.; Guruge, K. S.; Kannan, K.; Yamashita, N. Perfluoroalkyl substances in the blood of wild rats and mice from 47 prefectures in Japan: use of samples from nationwide specimen bank. *Arch. Environ. Contam. Toxicol.* **2013**, *65* (1), 149–70.

(40) Newton, S.; McMahan, R.; Stoeckel, J. A.; Chislock, M.; Lindstrom, A.; Strynar, M. Novel polyfluorinated compounds identified using high resolution mass spectrometry downstream of manufacturing facilities near Decatur, Alabama. *Environ. Sci. Technol.* **2017**, *51* (3), 1544–1552.

(41) Strynar, M.; Dagnino, S.; McMahan, R.; Liang, S.; Lindstrom, A.; Andersen, E.; McMillan, L.; Thurman, M.; Ferrer, L.; Ball, C. Identification of novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) in natural waters using accurate mass time-of-flight mass spectrometry (TOFMS). *Environ. Sci. Technol.* **2015**, *49* (19), 11622–30.

(42) Lin, Y.; Ruan, T.; Liu, A.; Jiang, G. Identification of novel hydrogen-substituted polyfluoroalkyl ether sulfonates in environmental matrices near metal-plating facilities. *Environ. Sci. Technol.* **2017**, *51* (20), 11588–96.

- (43) Rotander, A.; Karrman, A.; Toms, L. M.; Kay, M.; Mueller, J. F.; Gomez Ramos, M. J. Novel fluorinated surfactants tentatively identified in firefighters using liquid chromatography quadrupole time-of-flight tandem mass spectrometry and a case-control approach. *Environ. Sci. Technol.* **2015**, *49* (4), 2434–42.
- (44) Jin, H.; Zhang, Y.; Zhu, L.; Martin, J. W. Isomer profiles of perfluoroalkyl substances in water and soil surrounding a Chinese fluorochemical manufacturing park. *Environ. Sci. Technol.* **2015**, *49* (8), 4946–54.
- (45) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying small molecules via high resolution mass spectrometry: communicating confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097–8.
- (46) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Bioconcentration and tissue distribution of perfluorinated acids in rainbow trout (*oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **2003**, *22* (1), 196–204.
- (47) Martin, J. W.; Mabury, S. A.; Solomon, K. R.; Muir, D. C. G. Dietary accumulation of perfluorinated acids in juvenile rainbow trout (*oncorhynchus mykiss*). *Environ. Toxicol. Chem.* **2003**, *22* (1), 189–95.
- (48) Conder, J. M.; Hoke, R. A.; De Wolf, W.; Russell, M. H.; Buck, R. C. Are PFCA's bioaccumulative? A critical review and comparison with regulatory criteria and persistent lipophilic compounds. *Environ. Sci. Technol.* **2008**, *42* (4), 995–1003.
- (49) USEPA. *Long-Chain Perfluorinated Chemicals Action Plan*; United States Environmental Protection Agency (USEPA): Washington, DC, 2009.
- (50) Shi, G.; Cui, Q.; Pan, Y.; Sheng, N.; Sun, S.; Guo, Y.; Dai, J. 6:2 Chlorinated polyfluorinated ether sulfonate, a PFOS alternative, induces embryotoxicity and disrupts cardiac development in zebrafish embryos. *Aquat. Toxicol.* **2017**, *185*, 67–75.
- (51) Wang, Y.; Vestergren, R.; Shi, Y.; Cao, D.; Xu, L.; Cai, Y.; Zhao, X.; Wu, F. Identification, Tissue Distribution, and Bioaccumulation Potential of Cyclic Perfluorinated Sulfonic Acids Isomers in an Airport Impacted Ecosystem. *Environ. Sci. Technol.* **2016**, *50* (20), 10923–10932.
- (52) De Silva, A. O.; Spencer, C.; Scott, B. F.; Backus, S.; Muir, D. C. Detection of a cyclic perfluorinated acid, perfluoroethylcyclohexane sulfonate, in the Great Lakes of North America. *Environ. Sci. Technol.* **2011**, *45* (19), 8060–6.
- (53) de Solla, S. R.; De Silva, A. O.; Letcher, R. J. Highly elevated levels of perfluorooctane sulfonate and other perfluorinated acids found in biota and surface water downstream of an international airport, Hamilton, Ontario, Canada. *Environ. Int.* **2012**, *39* (1), 19–26.
- (54) Houde, M.; Douville, M.; Despatie, S. P.; De Silva, A. O.; Spencer, C. Induction of gene responses in St. Lawrence River northern pike (*Esox lucius*) environmentally exposed to perfluorinated compounds. *Chemosphere* **2013**, *92* (9), 1195–200.
- (55) Lescord, G. L.; Kidd, K. A.; De Silva, A. O.; Williamson, M.; Spencer, C.; Wang, X.; Muir, D. C. Perfluorinated and polyfluorinated compounds in lake food webs from the Canadian high Arctic. *Environ. Sci. Technol.* **2015**, *49* (5), 2694–702.
- (56) Barzen-Hanson, K. A.; Roberts, S. C.; Choyke, S.; Oetjen, K.; McAlees, A.; Riddell, N.; McCrindle, R.; Ferguson, P. L.; Higgins, C. P.; Field, J. A. Discovery of 40 classes of per- and polyfluoroalkyl substances in historical aqueous film-forming foams (AFFFs) and AFFF-impacted groundwater. *Environ. Sci. Technol.* **2017**, *51* (4), 2047–2057.
- (57) Baduel, C.; Mueller, J. F.; Rotander, A.; Corfield, J.; Gomez-Ramos, M. J. Discovery of novel per- and polyfluoroalkyl substances (PFASs) at a fire fighting training ground and preliminary investigation of their fate and mobility. *Chemosphere* **2017**, *185*, 1030–1038.
- (58) Dinizon, I. K.; Trier, X.; Frömel, T.; Helmus, R.; Knepper, T. P.; de Voogt, P. High resolution mass spectrometry of polyfluorinated polyether-based formulation. *J. Am. Soc. Mass Spectrom.* **2016**, *27* (2), 309–318.
- (59) Gebbink, W. A.; van Asseldonk, L.; van Leeuwen, S. P. J. Presence of emerging per- and polyfluoroalkyl substances (PFASs) in river and drinking water near a fluorochemical production plant in the Netherlands. *Environ. Sci. Technol.* **2017**, *51* (19), 11057–11065.
- (60) Langlois, I.; Oehme, M. Structural identification of isomers present in technical perfluorooctane sulfonate by tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2006**, *20* (5), 844–50.
- (61) Heydebreck, F.; Tang, J.; Xie, Z.; Ebinghaus, R. Alternative and Legacy Perfluoroalkyl Substances: Differences between European and Chinese River/Estuary Systems. *Environ. Sci. Technol.* **2015**, *49* (14), 8386–95.
- (62) Sun, M.; Arevalo, E.; Strynar, M.; Lindstrom, A.; Richardson, M.; Kearns, B.; Pickett, A.; Smith, C.; Knappe, D. R. U. Legacy and emerging perfluoroalkyl substances are important drinking water contaminants in the Cape Fear River watershed of North Carolina. *Environ. Sci. Technol. Lett.* **2016**, *3* (12), 415–419.
- (63) Pretsch, E.; Bühlman, P.; Badertscher, M. *Summary Tables. In Structure Determination of Organic Compounds: Tables of Spectral Data*; Springer Science & Business Media: Secaucus, NJ, 2009; pp 35–39.
- (64) Ricci, A.; Piccolella, S. From the collisionally induced dissociation to the enzyme-mediated reactions: the electron flux within the lignan furanic ring. In *Tandem Mass Spectrometry - Applications and Principles*; Prasain, J. K., Ed.; InTech: Rijeka, Croatia, 2012.
- (65) Alsmeyer, Y. W.; Childs, W. V.; Flynn, R. M.; Moore, G. G.; Smeltzer, J. C. Electrochemical fluorination and its applications. In *Organofluorine Chemistry: Principles and Commercial Applications*, 1 ed.; Banks, R. E.; Smart, B. E.; Tatlow, J. C., Eds. Springer: New York, pp 131–135.
- (66) Burton, D. J.; Chambers, R. D.; Drakesmith, F. G.; Hutchinson, J.; Kitazume, T.; Lu, L.; Percy, J. M.; Sandford, G.; Yamazaki, T. *Organofluorine Chemistry: Techniques and Synthesis*, 1 ed.; Springer: Berlin Heidelberg, 1997.
- (67) Spahn, D. R. Blood substitutes. Artificial oxygen carriers: perfluorocarbon emulsions. *Crit. Care* **1999**, *3* (5), R93–7.
- (68) Seghatchian, J.; de Sousa, G. An overview of unresolved inherent problems associated with red cell transfusion and potential use of artificial oxygen carriers and ECO-RBC: current status/future trends. *Transfus. Apher. Sci.* **2007**, *37* (3), 251–259.
- (69) Bauer, J.; Zahres, M.; Zellermann, A.; Kirsch, M.; Petrat, F.; de Groot, H.; Mayer, C. Perfluorocarbon-filled poly(lactide-co-glycolide) nano- and microcapsules as artificial oxygen carriers for blood substitutes: a physico-chemical assessment. *J. Microencapsulation* **2010**, *27* (2), 122–32.
- (70) 3M. Fluorinert Electronic Liquid FC-43. In *3M Performance Materials*; 3M: Maplewood, Minnesota, 2000.
- (71) 3M. Electronic Liquids. http://solutions.3m.com/wps/portal/3M/en_US/Electronics_NA/Electronics/Products/Electronics_Product_Catalog/~/_Electronic-Liquids?N=8704931 (accessed April 4, 2017).
- (72) Hong, A. C.; Young, C. J.; Hurley, M. D.; Wallington, T. J.; Mabury, S. A. Perfluorotributylamine: A novel long-lived greenhouse gas. *Geophys. Res. Lett.* **2013**, *40* (22), 6010–6015.
- (73) Demarque, D. P.; Crotti, A. E. M.; Vescechi, R.; Lopes, J. L. C.; Lopes, N. P. Fragmentation reactions using electrospray ionization mass spectrometry: an important tool for the structural elucidation and characterization of synthetic and natural products. *Nat. Prod. Rep.* **2016**, *33* (3), 432–455.
- (74) Xu, G.; Huang, T.; Zhang, J.; McClure, T. D.; Miao, S. Study of free radical fragment ions generated from ESI-CID-MS-MS Using LTQ and LTQ Orbitrap mass spectrometers. <http://www.chromatographyonline.com/study-free-radical-fragment-ions-generated-esi-cid-ms-ms-using-ltq-and-ltq-orbitrap-mass-spectrometry> (accessed Aug 4, 2017).
- (75) Kind, T.; Fiehn, O. Advances in structure elucidation of small molecules using mass spectrometry. *Bioanal. Rev.* **2010**, *2* (1), 23–60.
- (76) Ghulam Musharraf, S.; Goher, M.; Hussain, A.; Choudhary, M. I. Electrospray tandem mass spectrometric analysis of a dimeric conjugate, salivariolone and related compounds. *Chem. Cent. J.* **2012**, *6* (1), 120.

- (77) Holcapek, M.; Jirasko, R.; Lisa, M. Basic rules for the interpretation of atmospheric pressure ionization mass spectra of small molecules. *J. Chromatogr. A* **2010**, *1217* (25), 3908–21.
- (78) Thurman, E. M.; Ferrer, I.; Pozo, O. J.; Sancho, J. V.; Hernandez, F. The even-electron rule in electrospray mass spectra of pesticides. *Rapid Commun. Mass Spectrom.* **2007**, *21* (23), 3855–3868.
- (79) Wilfried, M. A.; Ricardo, A.; Correa, C. *Interpretation of MS-MS mass spectra of drugs and pesticides*; Wiley: Hoboken, NJ, 2017.
- (80) Jackson, A. H.; Jackson, A. H.; Artico, M.; Anderson, H. J.; Loader, C. E.; Gossauer, A.; Nesvadba, P.; Dennis, N. Reactivity of the 1H-Pyrrole Ring System. In *Reactivity of the 1H-Pyrrole Ring System, in Chemistry of Heterocyclic Compounds: Pyrroles, Part One: The Synthesis and the Physical and Chemical Aspects of the Pyrrole Ring*; Jones, R. A., Ed.; John Wiley & Sons, Inc.: Hoboken, NJ, 1990; Vol. 48, pp 460–462.
- (81) Tucci, F. C.; Zhu, Y.-F.; Guo, Z.; Gross, T. D.; Connors, P. J., Jr; Struthers, R. S.; Reinhart, G. J.; Wang, X.; Saunders, J.; Chen, C. A novel synthesis of 7-aryl-8-fluoro-pyrrolo[1,2-a]pyrimidin-4-ones as potent, stable GnRH receptor antagonists. *Bioorg. Med. Chem. Lett.* **2002**, *12* (23), 3491–3495.
- (82) Wilkerson, W. W.; Galbraith, W.; Gans-Brangs, K.; Grubb, M.; Hewes, W. E.; Jaffee, B.; Kenney, J. P.; Kerr, J.; Wong, N. Anti-inflammatory 4,5-Diarylpyrroles: Synthesis and QSAR. *J. Med. Chem.* **1994**, *37* (7), 988–998.
- (83) Ruebsam, F.; Webber, S. E.; Tran, M. T.; Tran, C. V.; Murphy, D. E.; Zhao, J.; Dragovich, P. S.; Kim, S. H.; Li, L.-S.; Zhou, Y.; Han, Q.; Kissinger, C. R.; Showalter, R. E.; Lardy, M.; Shah, A. M.; Tsan, M.; Patel, R.; LeBrun, L. A.; Kamran, R.; Sergeeva, M. V.; Bartkowski, D. M.; Nolan, T. G.; Norris, D. A.; Kirkovsky, L. Pyrrolo[1,2-b]pyridazin-2-ones as potent inhibitors of HCV NS5B polymerase. *Bioorg. Med. Chem. Lett.* **2008**, *18* (12), 3616–3621.
- (84) Black, B. C.; Hollingworth, R. M.; Ahammadsahib, K. I.; Kukel, C. D.; Donovan, S. Insecticidal Action and Mitochondrial Uncoupling Activity of AC-303,630 and Related Halogenated Pyrroles. *Pestic. Biochem. Physiol.* **1994**, *50* (2), 115–128.
- (85) Gribble, G. W.; Roy, S.; Roy, S. Fluorinated Imidazoles and Benzimidazoles. In *Fluorine in Heterocyclic Chemistry*, Vol. 1; Nenajdenko, V., 2014; pp 323–367.
- (86) Carini, D. J.; Chiu, A. T.; Wong, P. C.; Johnson, A. L.; Wexler, R. R.; Timmermans, P. B. M. W. M. The preparation of (perfluoroalkyl)imidazoles as nonpeptide angiotensin II receptor antagonists. *Bioorg. Med. Chem. Lett.* **1993**, *3* (5), 895–898.